IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Gleave Application No.: 10/646,436 Filed: 08/21/2003 Title: RNAi Probes Targeting Cancer-Related Proteins Attorney Docket No.: UBC-P-030 Customer No.: 57381	Group Art Unit:1635 Examiner: Kimberly Chong Confirmation No: 9171

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.131

I, Marina Larson declare as follows:

1. I am the attorney who prepared the application for ***RNAi probes targeting cancer-related proteins***, US Patent Application Serial No. 10/646,436 filed on August 21, 2003 and the provisionals from which it claims priority, Provisional Applications Nos. 60/405,193, filed Aug. 21, 2002, 60/408,152 filed Sep. 3, 2002, and 60/472,387, filed May 20, 2003.

2. The client in this matter is The University of British Columbia.

3. After the conception of the invention, but before the filing of Provisional Application No. 60/405,193, I was contacted by the inventors of this application to prepare a provisional application.

4. On or before July 18, 2002, I prepared a draft provisional application for this invention, a copy of which was sent to the client for review. A copy of this draft application is attached labeled July 18, 2002 Draft. This is a true and correct copy of a document from our files. As of this date, my files reflect that I possessed the data contained in Tables 1-4 of the filed provisional application and was beginning to prepare these tables.

5. On July 22, 2002, the client responded, giving the names of the inventors.

6. On August 1, 2002, the client responded to the draft application with comments.

7. On August 7, 2002, I received an article (DEMATTOS et al., *Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease*), from one of the inventors, providing additional information to consider in drafting the application.

8. On August 20, 2002, I revised the provisional application in view of comments received from the client. A true and correct copy of this application from our files is attached herein and labeled August 20, 2002 Draft. This draft contained all of the subject matter that was included in the submitted provisional application, with the exception Tables 5 and 6 of the filed application.

9. On August 21, 2002, I emailed the inventors regarding missing tables and further revised the application. Upon receipt of these I then filed the provisional application, which received serial number 60/405,193.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Marina L Larson

Marina Larson

4/22/09

Date

July 18, 2002 Draft

UBC.P-030-PV
Provisional Patent Application

RNAi PROBES TARGETING CANCER

Background of the Invention

This application relates to short double stranded RNAi probes useful in cancer therapy. RNA interference or "RNAi" is a term initially coined by Fire and co-workers to describe the observation that double-stranded RNA (dsRNA) can block gene expression when it is introduced into worms (Fire et al. (1998) Nature 391, 806-811, incorporated herien by reference). dsRNA directs gene-specific, post-transcriptional silencing in many organisms, including vertebrates, and has provided a new tool for studying gene function. RNAi involves mRNA degradation, but many of the biochemical mechanisms underlying this interference are unknown. The use of RNAi has been further described in Carthew et al. (2001) Current Opinions in Cell Biology 13, 244-248, and Elbashir et al. (2001) Nature 411, 494-498, both of which are incorporated herein by reference.

Within any given mRNA molecule, there are sites which are affected by RNAi probes, and sites which are not. Thus, one cannot simply chop up the overall sequence into subsequences of appropriate lengths (i.e., 21 to 23 bases pairs) to arrive at functional RNAi-based therapeutics. Indeed, published US Patent Application 2002-0086356-A1 discloses a method for use in assessing where target sites might be located in a mRNA sequence, although this method is not the only approach to development of effective RNAi sequences.

Summary of the Invention

The present invention provides RNAi sequences that are useful as therapeutics in the treatment of cancers of various types, including prostate cancer, renal cell carcinoma, breast cancer, bladder cancer, _____. These sequences target clusterin, IGFBP-5, IGFBP-2, IGF-Bis,_____.

Detailed Description of the Invention

The present invention relates to isolated RNA molecules (double-stranded; single-stranded) of from about 21 to about 23 nucleotides which mediate RNAi. That is, the

isolated RNAi of the present invention mediate degradation of mRNA of a gene to which the mRNA corresponds (mediate degradation of mRNA that is the transcriptional product of the gene, which is also referred to as a target gene). For convenience, such mRNA may also be referred to herein as mRNA to be degraded. The terms RNA, RNA molecule(s), RNA segment(s) and RNA fragment(s) may be used interchangeably to refer to RNA that mediates RNA interference. These terms include double-stranded RNA, single-stranded RNA, isolated RNA(partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA),as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the 21-23 nt RNA or internally (at one or more nucleotides of the RNA). Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. Collectively, all such altered RNAi are referred to as analogs or analogs of naturally-occurring RNA. RNA of 21-23 nucleotides of the present invention need only be sufficiently similar to natural RNA that it has the ability to mediate (mediates) RNAi. As used herein the phrase "mediates RNAi" refers to (indicates) the ability to distinguish which RNAI are to be degraded by the RNAi machinery or process. RNA that mediates RNAi interacts with the RNAi machinery such that it directs the machinery to degrade particular mRNAs. In one embodiment, the present invention relates to RNA molecules of about 21 to about 23 nucleotides that direct cleavage of specific mRNA to which their sequence corresponds. It is not necessary that there be perfect correspondence of the sequences, but the correspondence must be sufficient to enable the RNA to direct RNAi cleavage of the target mRNA.

A first group of RNA molecules in accordance with the present invention are directed to mRNA encoding clusterin, a protein also known as testosterone-repressed prostate message-2 (TRPM-2) or sulfated glycoprotein-2 (SGP-2). Clusterin is expressed in increased amounts by prostate tumor cells following androgen withdrawal. Furthermore, it has been determined that antisense therapy which reduces the expression of TRPM-2 provides therapeutic benefits in the treatment of cancer. In particular, such antisense therapy can be applied in

treatment of prostate cancer and renal cell cancer. (PCT Patent Publication WO 00/49937, which is incorporated herein by reference). Administration of therapeutic agents clusterin also can enhance sensitivity of cancer cells to chemotherapeutic agents and to radiotherapy both *in vitro* and *in vivo*. Sequences of specific RNA molecules which may be used to interfere with the expression of clusterin are listed in Table 1. (US Patent Application Serial No. 09/967,726 which is incorporated herein by reference) These sequences can be used alone or in combination with other chemotherapy agents in the treatment of prostate cancer, renal cell carcinoma and bladder cancer.

A second group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor binding protein-5 (IGFBP-5). It has been shown that inhibition of IGFBP-5 expression can delay the progression of hormone-regulated (prostatic or breast) tumor cells to hormone (e.g. androgen or estrogen) independence, provide a therapeutic method for the treatment of individuals, including humans, suffering from hormone regulated cancers, such as breast or prostate cancer and inhibit or delay the growth and metastatic progression of prostate, breast and other IGF-1 sensitive tumors in bone. (Published PCT Application No. WO01/05435, which is incorporated herein by reference.) These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 2. These sequences can be used alone or in combination with other chemotherapy agents.

A third group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor binding protein-2 (IGFBP-2). It has been shown that inhibition of expression of IGFBP-2 delays the progression of prostatic tumor cells to androgen independence, and provides a therapeutic benefit for mammalian individuals, including humans, suffering from hormone-regulated cancer such as prostate or breast cancer. In addition, the compositions of the invention can be used to inhibit or delay the growth and metastatic progression of such cancers. (Published PCT Application No. WO02/22642, which is incorporated herein by reference). These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 3. These sequences can be used alone or in combination with other chemotherapy agents.

A fourth group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor-_____ (IGF-Bis). Inhibition of expression of IGF-Bis _____ (is there a reference). These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 4. These sequences can be used alone or in combination with other chemotherapy agents.

other targets

The siRNA molecules of the invention are used in therapy to treat patients, including human patients, that have cancers of a type where a therapeutic benefit is obtained by the inhibition of expression of the targeted protein. **describe general protocol for therapeutic treatment. Is it in vivo, or ex vivo, or either, what kind of levels of treatment and frequency, etc?**

Example 1

Protocol for Transfection of LNCaP and PC3 cells with siRNA Duplexes

1) Cells preparation:
   In each well of 6-well plate seed 0.5 x106 of LNCaP cells [PC3 cell at the density 0.3 x106 per well] in appropriate media containing 5% FBS without antibiotics [penicillin/ streptomycin]
   Incubate the cells at 37 C in a humidified 5% CO2 incubator until they reach 40-50% confluence.
2) si RNA preparation:
   Prepare the following si RNA dilution in microcentrifuge tubes. For each well :
3) Prepare the following transfection reagent dilution in microcentrifuge tubes:
   For each well of 6-well plate dilute 4ml of OligoFECTAMINE Reagent into 11 ml of OPTI-MEM and incubate 10 min at room temperature.

4) Combine the diluted OligoFECTAMINE to the diluted si RNA duplexes and mix gently by inversion.

5) Incubate 20 min at room temperature.

6) Remove the media from the well and replace it with 800 ml of Opti-MEM.

7) Overlay the 200 ml of transfection complexes onto the cells.

8) Incubate 4 hrs at 37 degrees C in a CO_2 incubator.

9) add 500 ml of media containing 15 % FBS

10) after 24 hrs check gene expression by Real Time PCR or

11) check protein expression with Western Blot after 48 hours

August 20, 2002 Draft

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Provisional Patent Application

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Within any given mRNA molecule, there are sites which are affected by RNAi probes, and sites which are not. Thus, one cannot simply chop up the overall sequence into subsequences of appropriate lengths (i.e., 21 to 23 bases pairs) to arrive at functional RNAi-based therapeutics. Indeed, published US Patent Application 2002-0086356-A1 discloses a method for use in assessing where target sites might be located in a mRNA sequence, although this method is not the only approach to development of effective RNAi sequences.

Summary of the Invention

The present invention provides RNAi sequences that are useful as therapeutics in the treatment of cancers of various types, including prostate cancer, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, and melanoma. _____.
These sequences target clusterin, IGFBP-5, IGFBP-2, both IGF-BP-2 and 5 simultaneously, MITF, and B-raf_____.

Detailed Description of the Invention

The present invention relates to isolated RNA molecules (double-stranded;

single-stranded) of from about 21 to about 23 nucleotides which mediate RNAi. That is, the isolated RNAi of the present invention mediate degradation of mRNA of a gene to which the mRNA corresponds (mediate degradation of mRNA that is the transcriptional product of the gene, which is also referred to as a target gene). For convenience, such mRNA may also be referred to herein as mRNA to be degraded. The terms RNA, RNA molecule(s), RNA segment(s) and RNA fragment(s) may be used interchangeably to refer to RNA that mediates RNA interference. These terms include double-stranded RNA, single-stranded RNA, isolated RNA (partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA), as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the 21-23 nt RNA or internally (at one or more nucleotides of the RNA). Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. Collectively, all such altered RNAi are referred to as analogs or analogs of naturally-occurring RNA. RNA of 21-23 nucleotides of the present invention need only be sufficiently similar to natural RNA that it has the ability to mediate (mediates) RNAi. As used herein the phrase "mediates RNAi" refers to (indicates) the ability to distinguish which RNAi are to be degraded by the RNAi machinery or process. RNA that mediates RNAi interacts with the RNAi machinery such that it directs the machinery to degrade particular mRNAs. In one embodiment, the present invention relates to RNA molecules of about 21 to about 23 nucleotides that direct cleavage of specific mRNA to which their sequence corresponds. It is not necessary that there be perfect correspondence of the sequences, but the correspondence must be sufficient to enable the RNA to direct RNAi cleavage of the target mRNA.

A first group of RNA molecules in accordance with the present invention are directed to mRNA encoding clusterin, a protein also known as testosterone-repressed prostate message-2 (TRPM-2) or sulfated glycoprotein-2 (SGP-2). Clusterin is expressed in increased amounts by prostate tumor cells following androgen withdrawal. Furthermore, it has been determined that antisense therapy which reduces the expression of TRPM-2 provides therapeutic benefits in the treatment of cancer. In particular, such antisense therapy can be applied in

treatment of prostate cancer and renal cell cancer. (PCT Patent Publication WO 00/49937, which is incorporated herein by reference). Administration of therapeutic agents clusterin also can enhance sensitivity of cancer cells to chemotherapeutic agents and to radiotherapy both *in vitro* and *in vivo*. Sequences of specific RNA molecules which may be used to interfere with the expression of clusterin are listed in Table 1. (US Patent Application Serial No. 09/967,726 which is incorporated herein by reference) These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts in the treatment of prostate cancer, renal cell carcinoma, bladder cancer, lung cancer, and melanoma.

A second group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor binding protein-5 (IGFBP-5). It has been shown that inhibition of IGFBP-5 expression can delay the progression of hormone-regulated (prostatic or breast) tumor cells to hormone (e.g. androgen or estrogen) independence, provide a therapeutic method for the treatment of individuals, including humans, suffering from hormone regulated cancers, such as breast or prostate cancer and inhibit or delay the growth and metastatic progression of prostate, breast and other IGF-1 sensitive tumors in bone. (Published PCT Application No. WO01/05435, which is incorporated herein by reference.) These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 2. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts.

A third group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor binding protein-2 (IGFBP-2). It has been shown that inhibition of expression of IGFBP-2 delays the progression of prostatic tumor cells to androgen independence, and provides a therapeutic benefit for mammalian individuals, including humans, suffering from hormone-regulated cancer such as prostate or breast cancer. In addition, the compositions of the invention can be used to inhibit or delay the growth and metastatic progression of such cancers. (Published PCT Application No. WO02/22642, which is incorporated herein by reference). These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 3. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment

concepts.

A fourth group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor-2 and 5 simultaneously (IGF-Bis). Inhibition of expression of both IGFBP-2 and IGFBP-5 can delay the progression of hormone-regulated (prostatic or breast) tumor cells to hormone (e.g. androgen or estrogen) independence, provide a therapeutic method for the treatment of individuals, including humans, suffering from hormone regulated cancers, such as breast or prostate cancer and inhibit or delay the growth and metastatic progression of prostate, breast and other IGF-1 sensitive tumors in bone potentially more effectively than the inhibition of either of these factors (Published PCT Application No. WO01/05435, Published PCT Application No. WO02/22642, AND XXXXXX – Martin's IGFBP-2/-5 PROVISIONAL BISPECIFIC ANTISENSE SUBMISSION which are incorporated herein by reference.) These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 4. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts.

A fifth group of RNA or DNA antisense molecules in accordance with the present invention are directed to mRNA encoding the group of microphthalmia transcription factors (Mitf). Bcl-2 is regulated in melanoma and other cells by the master regulator Mitf which has been reported to modulate melanoma cell viability, lineage survival, and susceptibility to apoptosis (McGill et al. (2002) Cell 109, 707-718, incorporated herein by reference). Mitf and Bcl-2 regulated by Mitf are expressed in increased amounts by various human tumors. RNAi or antisense therapy which reduces the expression of Mitf may provide therapeutic benefits in the treatment of cancer. (We propose that ?????) Mitf can also enhance sensitivity of cancer cells to chemotherapeutic agents and to radiotherapy both *in vitro* and *in vivo*. Sequences of specific antisense or RNA molecules which may be used to interfere with the expression of Mitf are listed in Table 5. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts in the treatment of melanoma, prostate cancer, renal cell carcinoma, bladder cancer, lung cancer, bone cancer and other tumors.

A sixth group of RNA or DNA antisense molecules in accordance with the present invention are directed to mRNA encoding BRAF. BRAF is a key player in cellular signal transduction and is activated by somatic missense mutations in 66% of malignant melanomas and at lower frequencies in a wide range of human cancers (Davies et al. (2002) Nature 417, 949-954, incorporated herein by reference). RNAi or antisense therapy which reduces the expression of activated and/or non-activated BRAF may provide therapeutic benefits in the treatment of cancer. (We propose that ?????) BRAF can also enhance sensitivity of cancer cells to chemotherapeutic agents and to radiotherapy both *in vitro* and *in vivo*. Sequences of specific antisense or RNA molecules which may be used to interfere with the expression of BRAF are listed in Table 6. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts in the treatment of melanoma, prostate cancer, renal cell carcinoma, bladder cancer, lung cancer, bone cancer and other tumors.

The siRNA molecules of the invention are used in therapy to treat patients, including human patients, that have cancers or other diseases of a type where a therapeutic benefit is obtained by the inhibition of expression of the targeted protein. siRNA molecules of the invention are administered to patients by one or more daily injections (intravenous, subcutaneous or intrathecal) or by continuous intravenous or intrathecal administration for one or more treatment cycles to reach plasma and tissue concentrations suitable for the regulation of the targeted mRNA and protein.

Example 1

Protocol for Transfection of LNCaP and PC3 cells with siRNA Duplexes

1) Cells preparation:

In each well of 6-well plate seed 0.5 x 10^6 of LNCaP cells [PC3 cell at the density 0.3 x10^6 per well] in appropriate media containing 5% FBS without antibiotics [penicillin/ streptomycin]

Incubate the cells at 37 C . in a humidified 5% CO2 incubator until they reach 40-50% confluence.

2) si RNA preparation:

Prepare the following si RNA dilution in microcentrifuge tubes. For each well : 0.01-100 nM

3) Prepare the following transfection reagent dilution in microcentrifuge tubes:
For each well of 6-well plate dilute 4ml of OligoFECTAMINE Reagent into 11 ml of OPTI-MEM and incubate 10 min at room temperature.

4) Combine the diluted OligoFECTAMINE to the diluted si RNA duplexes and mix gently by inversion.

5) Incubate 20 min at room temperature.

6) Remove the media from the well and replace it with 800 ml of Opti-MEM.

7) Overlay the 200 ml of transfection complexes onto the cells.

8) Incubate 4 hrs at 37 degrees C in a CO_2 incubator.

9) add 500 ml of media containing 15 % FBS

10) after 24 hrs check gene expression by Real Time PCR or

11) check protein expression with Western Blot after 1, 6, 12, 24, 48, 72 and 96 hours